88* Detection of anaerobic bacteria in high numbers in sputum from Cystic Fibrosis patients with an acute exacerbation of pulmonary infection

T.F. Moriarty[1], J.S. Elborn[1], S. Patrick[1], G. Doring[2], M.M. Tunney[1]. [1]*Queen's University Belfast, Belfast, United Kingdom;* [2]*University of Tubingen, Tubingen, Germany*

Introduction and Aims: We have previously shown by culture that the lungs of clinically stable Cystic Fibrosis (CF) patients are not only colonised by commonly recognised aerobic bacteria, such as *Pseudomonas aeruginosa*, but also by a range of potentially pathogenic anaerobic species. The aim of this study was to determine whether anaerobes are also present in the sputum of CF patients with an acute exacerbation of pulmonary infection.
Methods: Sputum and mouthwash samples were collected, prior to commencing and at the end of antibiotic therapy, from 30 adult CF patients admitted for treatment of an acute exacerbation of pulmonary infection. Bacteria within the samples were detected by plating on selective agars, quantified by total viable count and identified by PCR and sequencing of 16S ribosomal RNA genes.
Results: Anaerobes from a range of species including *Prevotella, Veillonella, Propionibacterium, Actinomyces* and *Gemella* were detected in high numbers (up to 1×10^8 cfu/g of sputum) from all patients prior to commencing antibiotic treatment with the predominant aerobes (*P. aeruginosa* or *Burkholderia cepacia* complex) detected in similar or smaller numbers. Anaerobes were also detected in all patients at the end of antibiotic treatment but in the majority of patients they were present in lower numbers than detected before antibiotic treatment.
Conclusion: These results indicate that anaerobes are present within the CF lung during an acute exacerbation of pulmonary infection. Their presence could be of important clinical relevance to CF patients as they may contribute significantly to the inflammatory process.
Supported by: UK Cystic Fibrosis Trust (project grant PJ533).

89 Biofilm formation by anaerobic bacteria isolated from the sputum of patients with Cystic Fibrosis

T.R. Field[1], J.S. Elborn[2], M.M. Tunney[1]. [1]*School of Pharmacy, Queen's University Belfast, Belfast, United Kingdom;* [2]*School of Medicine and Dentistry, Queen's University Belfast, Belfast, United Kingdom*

Introduction and Aims: We have shown by culture that the lungs of Cystic Fibrosis (CF) patients are not only chronically infected with pathogens, such as *Pseudomonas aeruginosa*, but also by an array of other bacterial species, many of which are anaerobes [1]. As *P. aeruginosa* grows within a biofilm in the lungs of CF patients and as biofilm formation is considered an important virulence factor for CF pulmonary infection, this pilot study aimed to determine the ability of these anaerobes to form biofilms.
Methods: Biofilm formation by the predominant anaerobic species (*Prevotella, Veillonella, Propionibacterium* and *Actinomyces*) isolated from the sputum of CF patients, was assessed using a crystal violet micro-titre tray assay [2].
Results: Initial investigations with selected strains from each genera, in which the adhesion and biofilm times were varied, revealed that an adhesion time of 4 hours and a biofilm growth time of 24 hours were the optimal conditions for biofilm formation.
Using these parameters, the majority of the 42 isolates tested demonstrated the ability to form biofilms with significant biomass.
Conclusion: These results indicate that anaerobes present within the CF lung are capable of biofilm formation in vitro.

References

[1] Field, TR. et al. J Cyst Fibrosis 2006; 5(Suppl. 1): S15.
[2] Stepanovic, S. et al. J Microbiol Methods 2000; 40: 175–179.

90 Binding of A549 cells by clinical isolates of *P. aeruginosa*

H.C. Ryley, T. Buckle, J. Weeks. *Medical Microbiology, Cardiff University, Cardiff, United Kingdom*

Currently it is unknown if there are common infective factors related to the presence of "shared" strains of *Pseudomonas aeruginosa* isolated from some patients attending the same CF clinic. Here we report our study into a comparison of bacterial binding to a pneumocyte cell line, A549 by "shared" and unique clinical isolates and from immunocompromised non CF patients to explore the possibility of differing affinities in establishing lung infection.
Methods: Estimation of bacteria binding per cell was carried out following a 2 h bacterial exposure to a confluent layer of A549 cells as described by Simpson et al Infect Immunol 1992:60;3771–9. Shared strains are defined as isolates with same genotype carried by two or more patients. Representative isolates from 14 shared strains were used here and compared with 20 isolates with unique genotypes picked at random and 15 isolates from non CF patients. Binding of bacteria was allocated to one of three groups, no binding (<2 bacteria per cell), moderate binding (3–15 bacteria per cell) and high binding (>15 bacteria per cell).
Results: With the shared strains, 11 did not bind, 2 showed moderate binding and 1 high binding. 10 of the unique strains gave no binding, 6 moderate binding and 4 high binding. Despite these differences, Chi squared analysis indicated no significant difference between the two groups ($p < 0.2$). In the case of the non CF isolate, one gave no binding, moderate binding was seen with 5 and 9 gave high binding ($p > 0.05$).
Conclusions: There was no significant difference between shared and unique isolates in their affinity for A549 cells indicating that factors for increased transmissibility lie elsewhere. These results also indicate a trend in CF isolates towards low binding affinity to A549 cells and thus possibly to lung epithelium.

91 Variations in phenotype and virulence amongst isolates of a cystic fibrosis epidemic strain of *Pseudomonas aeruginosa*

J.L. Fothergill[1], C.A. Hart[1], S. Panagea[1], M.J. Walshaw[2], T.L. Pitt[3], C. Winstanley[1]. [1]*Medical Microbiology, University of Liverpool, Liverpool, United Kingdom;* [2]*Cardiothoracic Centre, Liverpool, United Kingdom;* [3]*Health Protection Agency, London, United Kingdom*

Infection by *Pseudomonas aeruginosa* plays an important role in the morbidity and mortality associated with cystic fibrosis (CF). In the Liverpool adult CF unit, it has been shown that patients infected with the transmissible Liverpool epidemic strain (LES) of *P. aeruginosa* suffer greater morbidity than those infected by non-LES strains. In some LES isolates, an unusual hypervirulence (HV) phenotype, characterized by over-expression of quorum-sensing (QS)-regulated virulence genes, has been identified.
Using assays for the QS-regulated exoproducts pyocyanin and LasA, we have demonstrated that the unusual HV phenotype is widespread amongst LES isolates. Sequential isolates from six individual patients revealed a wide variation in exoproduct secretion, antimicrobial susceptibility, morphology, motility, auxotrophy and hypermutability. Some LES isolates secreted no pyocyanin or LasA, due to mutations in the *lasR* gene. Isolates with the same colony morphology were found to have very different phenotypic characteristics. In addition, LES isolates exhibited variable virulence in a *Caenorhabditis elegans* infection model. These studies suggest that the unusual HV phenotype may play an important role in the greater morbidity of CF patients infected by the LES, and that LES isolates vary greatly in virulence and several phenotypic characteristics, including antimicrobial susceptibilities.
This work was undertaken under the framework of the UK CF Microbiology Consortium, an initiative funded by the Big Lottery Fund in association with the Cystic Fibrosis Trust.